TECOmedical Group

TECO®

2019-nCoV IgG Antibody assay

2019-nCoV IgG Antibody Assay

ELISA

Instructions for use
English

Research use only

Catalogue No. TE 1065

always your partner

Symbol Description

Kit Instructions

Lot Number

Expiry Date

Storage Temperature

Manufacturer

Keep away from sunlight

TE 1065

Attention

Intended use

Tests

Professional Use only
Not for first virus detection

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TECO® 2019-nCOV IgG Antibody Assay ELISA

CONT **Reagents and Materials Supplied:**

SYMBOL	DESCRIPTION	FORMAT
R1	**96-well plate Coated with nucleocapsid protein and spinous protein S1** 12 break apart strips of 8 wells (12 x 8 in total), in a frame with cover plate. Ready to use.	**1 plate**
R3	**Wash solution 20x**	**2 x 20 ml**
R4	**Sample dilution solution** Ready to use	**2 x 50 ml**
R2	**Conjugate** Ready to use	**1 x 12 ml**
R5	**Substrate solution** Ready to use	**1 x 12 ml**
R6	**Stop solution - 2M H_2SO_4** 2 M Sulphuric acid Ready to use 	**1 x 8 ml**
QC1	**Negative control** Ready to use	**2 x 1 ml**
QC2	**Positive control** Ready to use	**2 x 1 ml**
	**Kit instructions**.	**1 x**

Storage

Store kit at 2–8 °C in a dark place. Do not freeze.

Intended Use

The TECO® 2019-nCoV IgG Antibody Test ELISA is used for the qualitative detection of 2019-nCoV IgG antibodies in human serum or plasma samples

Limitations

IgG antibodies may not be detected in some patients with weakened immune function. The product can only be used for the detection of 2019-nCoV IgG antibody in human serum or plasma samples. **The test results obtained are for research use only.**

References

[1] Thomas G Ksiazek et al.
A novel coronavirus associated with severe acute respiratory syndrome
New England journal of medicine, 2003,348(20): 1953-1966.

[2] Raoul J de Groot et al.
Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Announcement of the Coronavirus Study Group.
Journal of Virology, 2013,87(14): 7790-7792.

[3] WHO
Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003
December 31, 2003

[4] WHO
Middle East respiratory syndrome coronavirus (MERS-CoV)
November 2019

[5] Chaolin Huang, Yeming Wang et al.
Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China
The Lancet, 2020

[6] National Health Committee of the People's Republic of China
Prevention and Control of New Coronavirus Pneumonia
As of 24:00, February 9, 2020

[7] Riou J, Althaus C L.
Pattern of early human-to-human transmission of Wuhan 2019-nCoV
bioRxiv, 2020
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Assay Principle

The TECO® 2019-nCoV IgG Antibody Test (TE1065) is an indirect ELISA using a nucleocapsid proteins, spinous protein S1 labeled plate and IgG enzyme-labeled antibody for detection. The nucleocapsid/spinous S1 proteins and IgG enzyme-labeled antibody form a complex with the 2019-nCoV IgG antibodies present in the sample, followed by a substrate color reaction.

Materials Required and not Supplied

- Pipettes 5µl – 500 µl
- Multichannel pipettes 50 - 300 µl
- Graduated cylinders for reconstitution or diluting reagents
- Manual aspiration system or automated washer for ELISA plates
- Aqua dest or ultrapure water
- Vortex mixer
- Incubator 37°C
- ELISA plate reader for 96 wells formats and capable of measuring 450 nm
- (reference 620 - 630 nm)
- Software package for data generation and analysis

Warnings and precautions

This kit is intended for research use by professional persons only.
The kit is not intended for first virus detection
Follow the instructions carefully.
Observe expiry dates stated on the labels. Use reagents within 30 minutes after opening of the kit. Refer to" Materials Safety Data Sheet" for more detailed safety information.

Material of animal origin used in the preparation of this kit has been obtained from animals certified as healthy, but these materials should be handled as potentially infectious.

Material of human origin used in the preparation of this kit has been tested and found non-reactive for HIV-1 and HIV-2 as well as for HCV antibodies and HbsAg but should, nonetheless, be handled as potentially infectious.

TECOmedical AG is not liable for loss or harm caused by non-observance of the kit instructions.

1. For research use only
2. Treat all specimen samples as potentially biohazardous material.
   Follow General Precautions when handling contents of this kit and any patient samples.
3. Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements.
4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Store assay reagents as indicated. The substrate solution should be protected from light and oxidation. When the substrate solution changes from colorless to light blue, the reagent is invalid and should be discarded
6. Do not use coated strips if pouch is punctured.
7. Test each sample in duplicate.
8. Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of liquids.

9. a. 2M H_2SO_4 is caustic and can be harmful for skin, eyes, and mucosae.
   b. Handle TMB with care. Do not ingest. Avoid contact with skin, eyes, or clothing. Should there be any contact, wash with water. If ingested, call a physician.
10. A mercury-free preservative is used. Incidental contact with or ingestion of buffer solutions may cause irritation of skin, eyes, or mouth. Should there be any contact, wash with water. If ingested, call a physician.

Preparation of reagents

R1 96-well plate coated with nucleocapsid protein and spinous protein S1

12 break apart strips of 8 wells (96 in total) in a frame and sealed in a foil bag.
Fit strip wells firmly into the frame. After opening, return any unused wells to the original foil package and seal.
Store at 2–8°C until expiration date.
Cover for microtiter plates

R3 Wash solution 20x

2 vial of 20 ml Wash Buffer concentrate. Dilute the 1:20 concentrate with deionized or distilled water up to 400 ml.
Store undiluted at 2–8°C until expiration date. If crystals appear in the concentrated washing solution, dissolving in a 30°C water bath
The diluted washing solution is stable for 2 weeks at 2–8 °C.

R4 Sample dilution solution

2 vials of 50 ml, ready to use.
Store at 2–8 °C until expiration date.

R2 Conjugate

Anti-human IgG antibodies, conjugated with HRP; stabilized with protein stabilization solution
1 vial of 12 ml, ready to use.
Store at 2–8 °C until expiration date.

R5 Substrate solution

1 vial of 12 ml of H_2O_2 stabilized tetramethylbenzidine.
Ready to use. Store at 2–8°C until expiration date.

R6 Stop solution - 2M H_2SO_4

1 vial of 8 ml of 2M Sulphuric acid.
Ready to use. Store at 2–8°C until expiration date.

QC1 Negative Control

2 vials of 1 ml
Ready to use. Store at 2–8°C until expiration date.

QC2 Positive Control

2 vials of 1 ml
Ready to use. Store at 2–8°C until expiration date.

Preparation and stability of samples

Collect samples according to standard laboratory procedures. Avoid cross- contamination among samples. Sample labeling should be clear and correct without mistake.

Sample Type

The assay is validated for serum and plasma.

Sample transportation

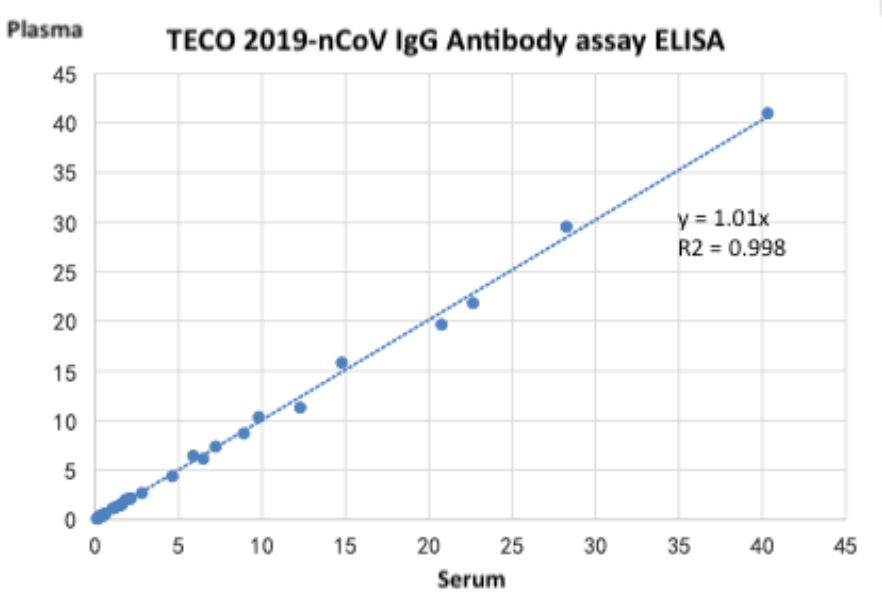
Sample transportation should comply with national biosafety requirements

Stability

Maximum 3 days at 2-8°C
Maximum 1 month at -20°C
Longer storage at -80°C
Maximum 5 freeze/thaw cycles

Serum - EDTA Plasma

A regression analysis for corresponding plasma/serum samples gave the following results:
Plasma value = 1.01 x Serum value with a correlation of r =0.998



Sample Handling

Samples must be diluted 1:200 with Sample dilution solution **R4** before pipetting into the wells. (e.g. 5 µl sample with 995 µl Sample dilution solution).

Assay procedure

All determinations (controls and samples) should be assayed in duplicate. When performing the assay, the controls and samples should be pipetted as fast as possible (<15 minutes).
To avoid distortions due to differences in incubation times, the Conjugate, Substrate Solution and Stop Solution should be added to the plate in the same order and with the same time interval as the samples. A multichannel pipette is essential.

Allow all reagents to stand at room temperature (20–25°C) for at least 30 minutes. During all incubation steps, plates should be sealed with the adhesive foil or a plastic cover. For light protection, incubate in a dark incubator or cover plate with aluminum foil.

1. Allocate the wells of the Microtiter plate **R1** for controls and samples, use the first well for the blank
2. Dilute samples 1:200 with Sample dilution solution **R4** (e.g. 5 µl sample with 995 µl Sample dilution solution). Dilution for automatic ELISA Platforms see below.
3. Pipette 100 µl of each ready to use control (**QC1** and **QC2**) and each diluted sample into the corresponding wells
4. Cover the wells with the plastic cover and incubate the plate for 25 ± 2 min at 37°C
5. After incubation, aspirate the wells by using a plate washer or manually decant by inverting the plate. Wash the wells 3 times with 300 µl diluted Washing solution per well. Wait 40 seconds after adding the Washing solution. After the last wash cycle tap the inverted wells on a dry absorbent surface to remove excess wash solution use of an automatic plate washer is recommended
6. Following the last washing step, pipette 100 µl of conjugate **R2** into each well (multichannel pipette)
7. Cover the wells with a plastic cover and incubate the plate for 25 ± 2 min at 37°C
8. After incubation wash the wells 3 times with Washing solution as described in step 5
9. Pipette 100 µl of the Substrate Solution **R5** into each well (multichannel pipette)
10. Incubate the plate in the dark, for 10 ± 1 min at 37°C
11. Stop the reaction by adding 50 µl of Stop Solution **R6** (multichannel pipette). After mixing, measure the color reaction within 5 minutes at 450 nm (reference wavelength between 620 / 630 nm)

Automatic ELISA platforms (e.g. DS2, DSX)

Pipette 15 µl sample + 285 µl Sample dilution solution **R4** in the deep well. Mix and take 10 µl directly into the microtiter plate **R1** with 90 µl Sample dilution solution **R4**

Calculation of results

The index (I) is used to determine whether the test sample contains the 2019-nCoV IgG antibody

Calculation steps of the index (I):

- Calculate the average value of OD of two positive controls and the average value of OD of two negative controls.
- Calculate the index (I) of the positive and negative controls and the samples to be tested as follows:

$$\text{Index Value Control/Sample} = \frac{\text{Average OD of control or OD of sample}}{\text{Average OD of positive control x 0.5 plus Average OD of negative control}}$$

- The index (I) value of positive control should be 1.2-2.5, and the index (I) value of negative control should be less than or equal to 0.4
- Index (I) value sample < 0.5 Sample is judged as negative
- Index (I) value sample ≥ 0.5 Sample is judged as positive

For each assay, the results of the controls must be within the above-mentioned target range. If control values are not within the limits of the target range, the assay results should be considered questionable and the samples should be tested again

Reference Values

Positive rates of IgM and IgG antibody detection in different courses of COVID-19

Sampling period	Number of samples	IgM antibody %	IgG antibody %
Early (1-7 days)	54	72.22 (39/54)	59.26 (32/54)
Medium term (8-14 days)	44	93.18 (41/44)	90.01 (40/44)
Late (> 15 days)	155	95.48 (148/155)	98.71 (153/155)
Total	253	90.12 (228/253)	88.93 (225/253)

Sensitivity and specificity

	Sensitivity % (N = 253) (95% CI)	Specificity % (N=378) (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
IgM antibody	90.12 (85.37 – 92.89)	99.74 (98.52-99.95)	99.56 (97.20-99.98)	93.78 (90.57-95.66)
IgG antibody	88.93 (84.47-92.23)	99.74 (98.52-99.95)	99.56 (97.18-99.98)	91.36 (90.05-95.28)
IgM/IgG combination	95.26 (90.93-96.68)	99.47 (98.09-99.85)	99.81 (96.71-99.86)	96.91 (93.91-97.95)

Test performance

Precision
(Intra assay, N=75)

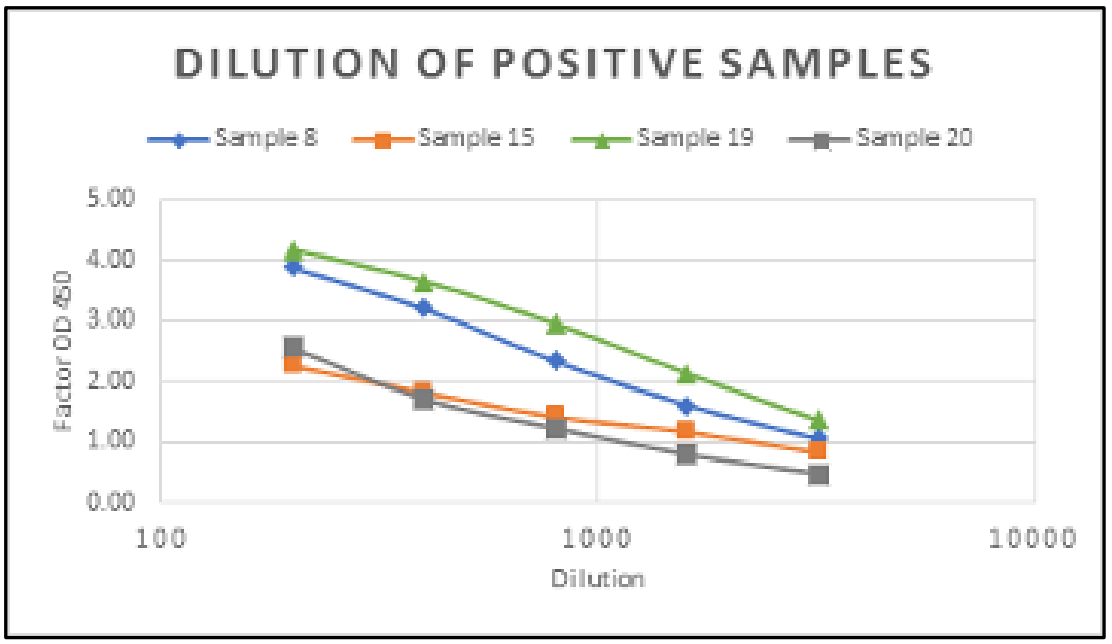
Sample	Mean (l)	SD	CV %
Positive control	4.80	0.05	0.94
Medium Positive	3.20	0.09	2.92
Strong Positive	10.00	0.31	3.11

(Inter assay, N=75)

Sample	Mean (l)	SD	CV %
Positive control	4.80	0.06	1.23
Medium Positive	3.20	0.15	4.69
Strong Positive	10.00	0.50	5.00

Sample	Mean (I)	Number of results with I value ≥ 1.0	Number of results with I value < 1.0	Positive detection rate
Critical Positive	1.11	75	0	100%
Negative	0.20	0	75	0.00%
Negative control	0.12	0	75	0.00%

Sample dilution



Interference

Controlled test of potential interfering substances showed that here was no interference in the under-mentioned concentrations.

Substance	Concentration
Hemoglobin	7 mg/ml
Bilirubin	300 mg/l
Triglyceride	7.5 mmol/l
α-Interferon (Rhizoma)	4 mg/ml
Zanamivir	300 ng/ml
Ribavirin	100 mmol/l
Oseltamivir	1 mg/l

Cross reaction

Cross reaction was tested in human samples in three different batches of kits

Cross reactant	Number of test samples	Number of positive values (I value <1.0)
Coronavirus OC43	2	0
Coronavirus 229E	2	0
Coronavirus HKU1	2	0
Coronavirus NL63	2	0
Influenza type A H1N1	2	0
Influenza type A H3N2	1	0
Influenza type A H5N1	1	0
Influenza type A H7N9	1	0
Influenza type B Yamagata	2	0
Influenza type B Victoria	2	0
Measles (MAE)	2	0
Micoplasma pneumoniae	1	0
RSV	3	0
PIV	3	0
EB	2	0
ADV	2	0
HRV	2	0
EV68	2	0
hMPV	3	0

TECO® 2019-nCoV IgG Antibody Assay

Assay Procedure – Quick Guide

- Bring samples and reagents to room temperature (20-25°C) for 30 min. Mix the samples well.
- Wash Solution **R3** : Dilute 1:20 with deionized or ultrapure water.
- Dilute samples 1:200 with Sample Dilution Solution **R4**